THE PROTEIDS OF MUSCLE. By G. N. STEWART, M.A., D.Sc., M.D. and TORALD SOLLMANN, M.D.

Reprinted from the Journal of Physiology.
Vol. XXIV. No. 6, August 21, 1899.

WELLCOME INSTITUTE
LIBRARY
Coll. welMOmec
Coll. pam
No. WE 500
1899
S85t

22500887485

[*Reprinted from the Journal of Physiology.*
Vol. XXIV. No. 6, *August*, 1899.]

THE PROTEIDS OF MUSCLE.

By G. N. STEWART, M.A. D.Sc., M.D., *Professor*, and TORALD SOLLMANN, M.D., *Demonstrator of Physiology and Histology in the Western Reserve University, Cleveland, U.S.A.*

Historical Note. By G. N. Stewart.

This investigation was begun by me during my tenure of the George Henry Lewes Studentship, in the Cambridge Physiological Laboratory in November 1890, and continued in the Physiological Laboratory of the Harvard Medical School during 1894. A preliminary account of my results was given at a meeting of the Physiological Society on Jan. 21st, 1893, and a further communication on the same subject was read at a meeting of the American Physiological Society in June 1894. In these communications the following conclusions were formulated:

1. The substance in striped muscle which coagulates at or below 49° C. (the paramyosinogen of Halliburton) is, in general, present in extracts of muscle (made with 5 p.c. magnesium sulphate or 10 p.c. sodium chloride, or 10 p.c. ammonium chloride) in larger amount than the proteid which coagulates between 49° and 59° (the myosinogen of Halliburton).

2. Paramyosinogen is not simply carried down with myosinogen when muscle-plasma, obtained by cold and pressure, clots, or when muscle-extracts are precipitated by dilution, or by dilution and heating to 35°—40°, but is altered in the same way as myosinogen. Myosinogen is, accordingly, not more essential to the formation of the muscle-clot than paramyosinogen.

3. Saline and watery extracts of cardiac muscle contain, in general, the same proteids as similar extracts of skeletal muscle. But saline extracts of some hearts contain no myosinogen and a large amount of paramyosinogen. Such extracts yield precipitates on dilution and heating in a water-bath at 40°, of the same general character as those given by extracts of heart muscle containing both paramyosinogen and myosinogen, and as extracts of skeletal muscle.

4. From the heart muscle of the dog and rabbit a spontaneously coagulable plasma can be obtained by washing out the coronary vessels with cold normal saline solution, freezing the muscle, cutting it into thin

slices, expressing it, and filtering the expressed liquid in the cold. This is the case even when saline extracts of the same heart or of the spontaneously formed clot contain no myosinogen.

5. It is not possible to obtain pure paramyosinogen by adding 50 grm. of magnesium sulphate to 100 c.c. of a 5 p.c. magnesium sulphate extract of skeletal muscle and dissolving the precipitate in water. Only a small amount of proteid goes into solution in the water, and this consists of a mixture of paramyosinogen and myosinogen.

6. It is not possible by saturating with magnesium sulphate crystals a 5 p.c. magnesium sulphate extract of muscle, to completely free it from proteids coagulating below 60°.

7. By long-continued heating at 40° or even at lower temperatures it is possible to precipitate the whole of the paramyosinogen in an insoluble form either from its solutions or *in situ* in the muscle fibres. A distinct jelly-like coagulum is often produced without dilution in a 5 p.c. magnesium sulphate extract of muscle placed in a water-bath at 35°—40°.

8. The relative amount of paramyosinogen and myosinogen in extracts of one and the same skeletal muscle was found, within the limits of error of the quantitative methods used, the same in early rigor (a few hours after death), in well-established rigor (48 hours after death), and after rigor has passed off without putrefaction (7 days after death, in a room at a temperature a few degrees above freezing point). The resolution of rigor cannot, therefore, be due to the conversion of spontaneously coagulable myosinogen into non-coagulable paramyosinogen, an explanation which suggested itself not unnaturally at a time when solutions of pure paramyosinogen were supposed to be incapable of clotting at a temperature below that of ordinary heat-coagulation.

9. It seems, nevertheless, probable that paramyosinogen and myosinogen are not absolutely independent of each other; and it is possible that under certain conditions the one may be converted into the other.

10. By mechanical means (pressure with the fingers) such changes may be produced in fresh muscles that ordinary heat rigor (*i.e.* the rigor which takes place when a muscle is heated for a very short time to a temperature below the ordinary heat-coagulation temperature of paramyosinogen) can no longer be brought about, and this when the escape of liquid from the muscle has been entirely prevented. Here, although all the spontaneously coagulable constituents of the muscle are still within the anatomical muscle, the alteration of their normal position in the muscle fibres seems to hinder the development of the rigidity which, had they clotted in their normal situation, would have been produced. This experiment evidently has a bearing on the well-known fact that ordinary rigor, once established, can be broken down by mechanical means, and does not then recur.

11. Mere differences of coagulation temperature are not sufficient to characterise as chemical individuals the various proteids which have been described in muscle.

12. Albumose is absent from normal muscle extracts.

13. Hæmoglobin or myohæmatin is partly carried down even at the lowest temperature of heat-coagulation. It is not the case that the chief part of the hæmoglobin, as Halliburton states, is precipitated at 73° along with the serum-albumin.

14. The addition of potassium oxalate to muscle extracts does not prevent their coagulation.

At this point my investigations were interrupted for two years by other work. In the meantime the important papers of v. Fürth[1] appeared; and it seemed necessary before proceeding to the detailed publication of my results to take account of his conclusions, which in many respects agreed with my own, and to test certain of them by further work. I accordingly suggested to my friend and former pupil Dr T. Sollmann the advisability of working the subject over once more as a joint research. In the following pages it has not seemed worth while to indicate further than this note has already done the portions of the work for which I am alone responsible and those which we did together. The methods employed were very much the same all through, the main stress being laid on quantitative estimations of the various proteids. There are few subjects of research in which mere qualitative methods would seem more likely to mislead. I therefore do not consider it necessary to apologise for the publication of a portion of the considerable mass of quantitative results at my disposal even when they simply confirm conclusions arrived at by others in other ways. In my own estimations I determined separately (1) the proteid coagulating at 48·5° or under, (2) the proteid coagulating between 48·5° and 58·5°, (3) the proteid coagulating between 58·5° and 65°, and (4) the proteid coagulating between 65° and 80° or on boiling.

In our joint experiments we contented ourselves with the estimation of the first three precipitates only. For while muscle extracts yield a precipitate at about the temperature of coagulation of serum-albumin it is certain that some of this at least belongs to the lymph, and we do not know whether any of it belongs to the muscular fibres.

---

[1] *Arch. f. exp. Path. u. Pharmak.*, XXXVI. S. 231, 1895; and XXXVII. S. 389. 1896.

DETAILS OF THE METHOD USED, WITH INVESTIGATION OF SOME OF THE SOURCES OF ERROR.

Extraction of the muscle. A certain amount of the finely chopped muscle was weighed and the extrahent added to it usually in the proportion of 5 parts by volume of extrahent to 1 part by weight of muscle. In the earlier experiments the blood was first removed by washing out the vessels of a newly killed animal with normal saline solution, but in the later experiments, in which we were only concerned with paramyosinogen and myosinogen, this was deemed unnecessary, since no similar bodies occur in blood. The mixture of muscle and extrahent was allowed to stand, with occasional stirring, at a temperature always below 12° C., for 20 to 24 hours, then strained through linen. The residue was usually squeezed out strongly. The liquid was filtered through paper, and the determinations of the proteids made in the filtrate.

Arrangement for heat-precipitation. 50 or 100 c.c. of the solution was put into a beaker of about 120 c.c. capacity. A number of beakers (sometimes as many as ten) were usually heated at one time. The beakers were supported by rings in a large shallow tin basin containing 7 litres of water. It was heated by a rose-burner having a piece of sheet-iron rather larger than the bottom of the basin laid over it to distribute the heat. The beakers were immersed till almost the whole of their contents was below the surface of the water. A thermometer was supported in the bath, and another with a stirring rod was placed in each beaker. All the thermometers were corrected by comparison with a Charlottenburg standard thermometer. The temperature in the various beakers did not differ by more than a third of a degree. The liquid in them was frequently stirred. A slow rate of heating was adopted. Usually about 30 minutes were needed to bring the solution to the temperature at which the first precipitate came down. It was kept between this and the maximum temperature allowed for the particular proteid for from 20 to 30 minutes. The beakers were then removed from the bath and either filtered at once or left to settle for a few hours. The precipitates were collected on dried and weighed ashless filters, and washed, first with 20 to 40 c.c. of the extrahent, the washings being added to the filtrate; then with boiling distilled water until the washings gave no turbidity with barium chloride or silver nitrate (according to whether a solution of sulphate or chloride had been used as extrahent). The precipitates were next washed with 95 p.c. alcohol, dried at 110° to 120°, and weighed. In some cases the ash was determined and subtracted from the weight of the precipitate, but usually this was not thought necessary. The filtrate and first washings were again heated, and kept for 5 to 10 minutes at the maximum temperature corresponding to the first precipitate; then if no marked turbidity appeared, raised slowly to the temperature at which the next precipitate occurred, and treated as before.

Differences in the appearance of the precipitates. Great differences were noticed in the character of the precipitates and in the manner of filtration. But we cannot agree with Hewlett[1], that such differences in heat-precipitates are indicative of chemical differences; for we found that both paramyosinogen and myosinogen[2] usually coagulated in large flakes, but sometimes as a fine powder, sometimes as a dense turbidity, and sometimes, again, as a jelly-like clot. We agree in regard to this with v. Fürth[3], who in particular did not find the myosinogen precipitate "sticky," as Halliburton did. Palladin[4] observed similar variations in the case of vegetable proteids. His "plant-vitellin" in certain degrees of concentration came down as flakes, in others as a gelatinous mass.

Differences in filtration. Filtration was most difficult in the case of paramyosinogen, less difficult with myosinogen and easy with myoglobulin. Different extracts behaved very differently in regard to filtration, some filtering rapidly and quite clear from the start, others filtering slowly and needing repeated filtration to free them from turbidity. Greater difficulty was experienced in the case of ammonium chloride and sodium chloride extracts than with magnesium sulphate extracts. Extracts made with saturated magnesium sulphate, and therefore containing no paramyosinogen, usually filtered very readily. Occasionally it was impossible, in spite of all our efforts, to obtain a clear filtrate. The extract was then thrown away, and the determination abandoned. The difficulty in filtration could have been to a great extent obviated by the addition of even small quantities of acid; but in the actual determinations acid was never added, since it precipitates a portion of the muscle-proteids and alters the coagulation temperature of the rest. However, the reaction of all our extracts was acid, and remained acid throughout the determination.

Temperatures adopted for fractional heat-precipitation. In order to render our results comparable it was necessary to fix certain limits of temperature for each precipitate. The following table shows the temperatures at which the characteristic proteids in muscle extracts are precipitated according to various observers.

---

[1] This *Journal*, XIII. p. 510. 1892.

[2] We use Halliburton's terminology throughout the paper, although, as will be seen in the sequel, it appears to need a slight modification.

[3] *loc. cit.*

[4] *Zeitsch. f. Biol.* S. 191. 1895.

Animal	Muscle	Paramyosinogen		Turbidity or slight precip.	Myosinogen		Myo-globulin	Observer
		Turbidity	Flakes		Turbidity	Flakes		
Various	Various	40 to 47						Demant
,,	,,	40–43	45–50			55		Danilewski
Rabbit	Skeletal		47			56	63	Halliburton
,,	Thigh	42–44	45–49		52–55	56–59	63–65	The authors
Dog	,,	41–44	45–49	50–52	53–55	56–59	60–65	,, ,,
,,	Heart	40–47	47–50	50–51·5	55–56	56–59	62–66	,, ,,
Rabbit	Thigh	44–47	47–50		50–55	55–65		v. Fürth
Cat	,,		45–48	50–52		55–58	60–64	The authors
Frog	,,		45					Kühne
,,	,,	44–46	47–49	50	53–55	55–58	64	The authors
,,	Heart	44–46	47			51		,, ,,

The temperature limits which we finally adopted were for paramyosinogen up to 49·5°, for myosinogen 50° to 59°, for myoglobulin 60° to 65°. It must be remembered that the temperatures at which these precipitates occur are not sharply marked off from each other as the statements of some writers might lead one to suppose. Whilst there are certain fairly definite points where precipitation is specially prompt and abundant, some turbidity[1] or even a small precipitate, forms between the maxima, for instance in muscle extracts between 51° and 53°. We always counted this precipitate, when there was one, with the myosinogen, since the percentage differences between duplicate or triple determinations are less if this is done than if it is reckoned with the paramyosinogen. Exps. 1 and 2 illustrate this. Both were performed on 5 p.c. magnesium sulphate extracts. In Exp. 1, two, and in Exp. 2, three parallel determinations were made. In Exp. 1 the relative amount of the precipitate between 51° and 53° was exceptionally large.

	Experiment **1**.				Experiment **2**.			
	Temperature	*a*	*b*	Difference	*a*	*b*	*c*	Difference
Paramyosinogen	45°—49°	·0678	·0623	·0055	·2325	·2342	·2380	·0055
	51 —53	·0417	·0690		·0254	·0215	·0265	
Myosinogen	54 —60	·0678	·0540	·0138	·0489	·0515	·0544	·0055
Paramyosinogen	45 —53	·1095	·1313	·0218	·2579	·2557	·2645	·0088
Myosinogen	51 —60	·1095	·1230	·0135	·0743	·0730	·0809	·0066

---

[1] Very small differences in turbidity may be detected by comparing a test-tube containing the heated liquid with a control test-tube, containing the unheated liquid against a dull black surface. Where only small quantities of liquid are available, as in the case of extracts of the frog's heart, the comparison may easily be made with two narrow glass tubes of the same calibre. The tubes are laid side by side on a black surface and a black pencil-mark on a piece of white paper is viewed through them. The liquid may be heated in such tubes, the ends being closed by bits of rubber tubing stopped with glass rod.

As we could not find any difference, beyond the slight difference of coagulation temperature, between myosinogen and "myoglobulin," we are inclined to agree with v. Fürth that they are one and the same substance. But we preferred to estimate them separately, so that the results could be used by any who hold the view that they are distinct. In calculating the ratio of paramyosinogen to myosinogen (P : M) we have always added the myoglobulin to the myosinogen. The following table containing a few of our duplicate determinations taken at random will give an idea of the degree of accuracy of our method.

No of Exp.	Determination		Mean of	Maximum	Percentage		No. of Exp.	Determination		Mean of	Maximum	Percentage
	a	*b*	*a* and *b*	error	error			*a*	*b*	*a* and *b*	error	error
3	·1184	·1157	·1170	·0013	1·2	Paramy.	**4**	·0446	·0468	·0457	·0011	2·4
	·0313	·0313	·0313	0	0	Myosin.		·0119	·0167	·0143	·0024	16·1
	·0094	·0076	·0085	·0009	10·5	Myoglob.		·0148	·0185	·0166	·0019	11·4
	·2775[1]	·2703	·2736	·0036	1·3	Total		·0713	·0820	·0766	·0053	6·9
5	·0618	·0588	·0603	·0015	2	Myosin.	**6**	·1307	·1354	·1330	·0024	2
	·0118	·0160	·0139	·0021	18	Myoglob.		·0113	·0114	·0114	·0001	1
	·0736	·0748	·0742	·0006	1	Total		·1420	·1468	·1444	·0024	2
7	·0698	·0599	·0649	·0050	8	Paramy.	**8**	·0075[2]	·0038	·0057	·0019	33
	·0296	·0247	·0272	·0025	9	Myosin.		·0544	·0570	·0557	·0013	2
	·0175	·0171	·0173	·0002	1	Myoglob.		·0141	·0063	·0102	·0039	38
	·1169	·1017	·1094	·0077	7	Total		·0760	·0671	·0716	·0044	6

It will be seen that the method yields tolerably congruent results except where the quantity of the proteid is small, as in the case of the precipitate separating at 60° to 65°. For certain purposes, including those for which we used it, this quantitative method appears to be an adequate one, although, as everyone knows who has worked much at the estimation of proteids, any method in which precipitation by heat is employed leaves much to be desired. Whether the method of fractional heat-coagulation can be properly used for the characterisation and quantitative separation of proteids is another question, and has been the subject of prolonged discussion.

Halliburton has used the method in numerous researches[3], on the proteids of blood-serum, muscle, milk, &c. In all these he assumed that even minute differences in the temperature of coagulation, as determined under conditions with the degree of constancy attained in his experiments, furnished evidence of the probable existence of distinct proteid bodies. A similar line was taken by Corin and Bérard[1].

---

[1] In this Exp. the total proteid was determined by boiling the filtrate after separation of the myoglobulin. In the others the total includes only the substances coagulating below 65°.

[2] Here the paramyosinogen had doubtless for the most part undergone the change referred to hereafter as "spontaneous clotting."

[3] This *Journal*, v. p. 152, 1884; vii. p. 319, 1886; viii. p. 133, 1887; ix. p. 229, 1888; xi. p. 448, 1890; xiii. p. 806, 1892; xv. p. 90, 1894; and xviii. p. 306, 1895.

An energetic protest against the unguarded use of the method of fractional heat-coagulation was entered by Haycraft and Duggan[2], and by Haycraft[3]. They state that the coagulation temperature of proteids is raised by diluting their solutions. When, therefore, the greater part of a given proteid has been removed by heating the solution to the temperature at which coagulation first occurs, the remainder, being much more diluted, will not be precipitated until a higher temperature is reached. They also point out the possibility that during the heating, if it be prolonged, an alteration may be produced in the proteid which will raise its coagulation temperature.

Halliburton[4] in his paper on the proteids of milk, Hewlett[5] in a research carried on under Halliburton's guidance, and Frédericq[6], on behalf of Corin and Bérard, reply to the objections of Haycraft and Duggan. Hewlett asserts that the failure of dilute solutions to coagulate is due to an acid or alkaline reaction and consequent changes in the proteids, and not to simple dilution. Hewlett seems to attribute Haycraft and Duggan's results to their having added too much acid, Halliburton and Frédericq to their having added too little.

Several observers have investigated the influence of salts on the temperature of coagulation. Corin and Ansiaux[7] find that the presence of salts lowers the coagulation temperature. Varenne[8], however, stated that this is only true of some salts; others (*e.g.* magnesium sulphate in strengths of from 0·9 to 10 p.c. and sodium chloride 6 p.c.) raise it, while some (as sodium sulphate) have no effect. Limbourg[9] arrived at the conclusion that when the percentage of salt is increased within certain limits the coagulation temperature is first raised and then lowered. Halliburton asserts that 10 p.c.

---

[1] *Arch. de Biologie*, IX. p. 1.

[2] *Brit. Med. Journ.* p. 167. 1890.

[3] *Centralb. f. Physiol.* III., S. 474, 1890 ; *ibid.* Bd. IV. p. 1.

[4] This *Journal*, XI. p. 448. 1890.

[5] *Ibid.* XIII. p. 493. 1892.

[6] *Centralb. f. Physiol.* III. S. 601 and 633. 1890.

[7] *Bull. de l'Acad. roy. Belg.* XXI. 3e sér. p. 345. 1891.

[8] *Compt. rend.* CII. p. 129.

[9] *Zeitsch. f. phys. Chem.* XIII. p. 450. 1889.

sodium chloride depresses it, while 5 p.c. magnesium sulphate does not. This conclusion as regards magnesium sulphate is opposed to that of Ringer and Sainsbury[1], who find that magnesium sulphate lowers the coagulation temperature of the serum proteids. Rossbach[2] stated long ago that some alkaloids, and especially quinine, lower the temperature of coagulation of egg and muscle proteids.

To sum up the present state of opinion, we may say that practically everyone is agreed that the temperature of heat-coagulation of proteids may be modified by the reaction of the solvent and the presence of salts. According to some the coagulation temperature also depends on the degree of concentration of the proteids and the rate and duration of heating, the latter influence being possibly connected with changes produced in the proteid by the heating.

As to the applicability of the method of fractional heat-coagulation as a means of identifying and separating different proteids, all are agreed that when the coagulation temperatures fall far apart the method gives trustworthy results, or at least affords useful indications which ought to be followed up by other methods. But there is great difference of opinion as to its reliability when the differences in coagulation temperature only amount to two or three degrees, the weight of the evidence being, in our opinion, against it.

We have reinvestigated the effect of several of the alleged influences, but only as regards the coagulation of the muscle proteids, and we desire expressly to say that our results ought not to be indiscriminately applied to the coagulation of all proteid solutions.

The effect of acidulation of muscle extracts. All previous workers in this field are agreed that acids, including even acetic acid, produce a precipitate in muscle extracts. But while Halliburton states that myosinogen alone is so precipitated and paramyosinogen not, v. Fürth asserts that both are precipitated. v. Fürth's statement agrees with our own observations. The precipitate has a tendency to redissolve in an excess of acid. Further, we have found that not only does acidulation cause some of the proteid to be precipitated, but the coagulation temperature of the rest is lowered. This is particularly true of the paramyosinogen.

Thus in one experiment in an acidulated extract of frog's heart precipitation occurred as low as 25° to 30°. In an extract of rabbit's heart the first precipitate occurred before acidulation at 45° to 50°, after acidulation at 25° to 30°. These, to be sure, are extreme instances in which comparatively large quantities of acid were added. But even small quantities bring about distinct changes. Thus in 5 p.c. $MgSO_4$ extracts of skeletal muscle from three dogs, the temperature at which the first precipitate occurred fell after slight acidulation from 47 to 37, from 49 to 46, and from 51 to 48 respectively. Again, a 5 p.c. $MgSO_4$ extract of the thigh muscles of a dog became turbid at 48° and flaked at 55°. On adding to 25 c.c. of the extract five drops of 1 p.c. lactic acid, these temperatures became respectively 37° and 50°, and on adding to 25 c.c., 2 c.c. of 1 p.c. lactic acid, the extract flaked at 39°.

---

[1] This *Journal* XII. p. 170. 1891.

[2] Ref. in *Jahresber. f. Physiol.* (Hofmann and Schwalbe), I. S. 459. 1873.

Of the acids tried (in 1 p.c. solution) sulphuric acid had the greatest effect, then formic, acetic, and lactic acids, in this order. The following quantitative experiments illustrate the effect of acidulation on the amount of the various precipitates.

No. of Exp.	Temp. of coag.	Slightly acidulated	More acidulated	Difference due to greater acidulation	Proteid	No. of Exp.	Temp. of coag.	No acid	Acid	Difference
9	45°—48°	·2086	·1770	– ·0316	Paramy.	10	to 49°	·1528	·0866	– ·0662
	55 —58	·0959	·0578	– ·0381	Myosin.		49°—59°	·0430	·0735	+ ·0305
	58 —100	·0408	·0472	+ ·0064	Myoglob. and serum proteid		60 —65	0	·0175	+ ·0175
		·3453	·2820	– ·0633	Total			·1958	·1776	– ·0182

In Exp. 9 a 5 p.c. $MgSO_4$[1] extract of rabbit's thigh muscles was acidulated with dilute sulphuric acid. In Exp. 10, 50 c.c. of a 5 p.c. $MgSO_4$ extract of dog's thigh muscles was acidulated with 10 drops of 1 p.c. lactic acid. The estimation was made in both cases without filtering after acidulation.

We see that the total precipitable proteid (in originally moderately acid extracts) is decreased by acidulation, the brunt of this decrease falling on the paramyosinogen. The myosinogen may sometimes be apparently increased, presumably owing to the lowering of the coagulation temperature of the serum globulin. The changes are so great as to preclude the addition of any acid in quantitative work. Nevertheless it is absolutely necessary for the solution to have an acid or at least a neutral reaction. For, as Demant pointed out, an alkaline extract will not be precipitated at 47° even in several hours. Fortunately, however, the reaction of the extracts is usually freely acid. Since the degree of the natural acidity may be supposed to some extent to influence the temperature of coagulation, certain incidental observations on this point may be inserted here.

---

[1] $MgSO_4$ or "magnesium sulphate" always means in this paper the crystallized salt ($MgSO_4$, $7H_2O$).

Reaction of extracts of muscle. 1. Fresh aqueous or saline extracts of muscle are always acid or amphoteric.

2. The degree of acidity, in extracts of skeletal muscle, although variable, appears to be usually less than in extracts of cardiac muscle.

3. When extracts are allowed to stand, whether at a low temperature or at 37°, the acidity usually increases. Ultimately when putrefaction sets in, the reaction becomes alkaline (in 20 days in the cold; in one to three days at 37°).

4. In several extracts kept at 37°, the alkaline reaction resulting from putrefaction was again replaced by acidity in the course of about two days from the time at which the extract first became alkaline.

5. Dialysis caused an increase in acidity, coincident of course with the formation of a globulin precipitate.

6. After heat-precipitation of the paramyosinogen and myosinogen, the reaction was always more acid than before the heating.

The influence of salts on the temperature of coagulation. We did not make any special experiments on this subject, but we incidentally observed that magnesium sulphate when present to the amount of 5 p.c. slightly raises, and when present to the amount of 50 p.c. somewhat depresses the coagulation temperature of the muscle proteids.

The influence of the quantity of proteid in solution. On this point we performed a considerable number of experiments, both qualitative and quantitative, diluting the muscle extracts with known amounts of the extrahent. The following are specimens of our results obtained with extracts of the thigh muscles of dogs:

No. of Exp.	Degree of dilution	Temp. of 1st precip.	Temp. of 2nd precip.	Extrahent
11	undiluted	48·5°		5 parts by vol. of 10 p.c. ammonium chloride to 1 part by weight of muscle
	+ 5 vols. of extrahent	47		
12	undiluted	48·5°	turbid at 54° flakes at 58·4°	5 parts by vol. of 10 p.c. NaCl to 1 part by weight of muscle
	+ 3 vols. of extrahent	49	turbid at 51° flakes at 55°	
	+ 10 vols. of extrahent	47—50	turbid at 54·5° flakes at 56°—59°	

No. of Exp.	Proteid	Temp. of coagulation	Undiluted extract	Extract + 3 vols. extrahent	Difference due to dilution	Extract + 10 vols. extrahent	Difference due to dilution
13 Muscle extracted with 5 vols. 10 % NaCl	Paramyos.	to 49·5°	·2394	·2074	– ·0320	·2142	– ·0252
	Myosinogen	50°—59°	·1070	·1100	+ ·0030	·1200	+ ·0130
	Myoglobulin	60 —65	·0628	·0646	+ ·0018	·0476	– ·0152
	Serum proteid	66 —100	·0595	·0656	+ ·0061	·0920	+ ·0335
	Total		·4687	·4476	– ·0211	·4738	+ ·0051

In another experiment, in which a dog's thigh muscles were extracted with five volumes of 10 p.c. NH_4Cl solution, the paramyosinogen (the precipitate at and below 49·5°) in a measured quantity was ·7285 grm. In the same quantity of extract diluted with five volumes of the 10 p.c. NH_4Cl solution it was ·7222 grm., a difference of less than 1 p.c.

We conclude that, within the limits of error of our method, differences in the percentage proteid content have no appreciable effect on the temperature at which precipitation occurs, although there may be a slight effect on the completeness of precipitation, dilution rather favouring incomplete precipitation.

Experiments in which muscle was extracted for the same length of time with very different quantities of water or saline solutions gave similar results.

Influence of the duration of the heating. We will consider here only the influence on the amount of proteid precipitated, of varying the duration of the heating within moderate limits, with the view of finding for what length of time it is most advantageous to heat in order to obtain fairly constant and comparable results. Halliburton and most other workers have contented themselves with heating until flakes appear, filtering and reheating. If no precipitate occurs before or as soon as the original coagulation temperature is reached, they at once proceed to heat up to the temperature at which the next precipitate comes down. Haycraft's objection, that the second heating at the temperature of first coagulation has not usually been sufficiently prolonged, and that in consequence a portion at any rate of the second precipitate may really belong to the first, appears to us to be sound. So that if we heat a muscle extract for a very short time we run the risk of not precipitating all the paramyosinogen. Yet if we heat for a long time, we certainly run the risk of causing changes in the myosinogen which will determine its precipitation at an abnormally low temperature. The following experiment shows clearly that these dangers are not imaginary, but that the apparent quantities of the various proteids can be markedly altered by varying the duration of the heating. In particular, the increase in the ratio of the paramyosinogen to the myosinogen $\left(\frac{P}{M}\right)$ with the length of heating is very striking.

Exp. **14.** Dog's thigh muscles extracted with 5 volumes of 5 p.c. $MgSO_4$ solution. All determinations made in duplicate. The average difference between the corresponding duplicate observations was ·0054 grm. Only the means of the duplicates are quoted. Ten beakers containing the extract were put together into the bath. The first pair (*A*) were taken out immediately after the required temperatures were reached; the second pair (*B*) were left in 10 minutes after each temperature was reached; the third pair (*C*), half-an-hour; the fourth pair (*D*), an hour; the fifth pair (*E*), 2½ hours. The same length of time was consumed in raising each pair of beakers to the corresponding temperatures. In the columns *A—E*, *B—E*, &c. are given the differences between the amount of the various precipitates in *A* and *E*, &c. Opposite $\frac{P}{M}$ stands the ratio of the amount of paramyosinogen to that of myosinogen, the "myoglobulin" precipitate being reckoned with the myosinogen.

Temp.	*A*	*A—E*	*B*	*B—E*	*C*	*C—E*	*D*	*D—E*	*E*
to 49·5°	·1020	– ·0531	·1161	– ·0390	·1160	– ·0391	·1340	– ·0211	·1551
50°—59°	·0673	+ ·0167	·0517	– ·0010	·0540	+ ·0013	·0568	+ ·0041	·0527
60 —65	·0287	– ·0010	—	—	·0293	– ·0005	·0335	+ ·0043	·0297
66 —100	·0113	+ ·0011	—	—	·0069	– ·0033	·0121	+ ·0019	·0102
Total	·2035	– ·0403	—	—	·2083	– ·0345	·2323	– ·0015	·2438
$\frac{P}{M}$	1·06				1·39		1·48		1·88

The best that can be done under these circumstances is to adopt some arbitrary standard to render the results, as far as possible, comparable. As the figures for 10 minutes and for half-an-hour agreed most closely, we accepted 20 to 30 minutes as the best time.

THE PROPERTIES OF THE MUSCLE PROTEIDS.

We first attempted to obtain pure paramyosinogen and myosinogen by the methods of Halliburton.

We tried all his processes as carefully as possible, but failed to get the results which we had expected. The difficulties which we at first experienced in separating and washing the precipitates obtained by the methods of fractional precipitation with salts were overcome by means of the centrifuge, but when we attempted to redissolve them only a small portion went into solution, and in all cases the solutions so obtained gave precipitates both at or below 49·5°, and between 56° and 59°. As regards the method of obtaining myosinogen by heating a solution of the muscle-clot to 49° we met with no success, for the reason that when the muscle-clot is carefully separated from the serum it appears to be almost insoluble. v. Fürth was similarly disappointed in these methods, although he states that by methods of his own he succeeded in obtaining pure solutions. We did not attempt further to obtain the pure proteids, but determined to adopt the quantitative method we have described, our general idea being to estimate the various proteids before and after subjecting the extracts to the conditions which it was desired to study. Although a great part of the work in this line was completed before v. Fürth's researches were published, our results are, in the main, in accordance with his conclusions.

1. **Spontaneous precipitation.**

The general tendency of proteids to pass into insoluble, apparently more stable modifications, is well known. Even mechanical changes may determine this transformation. Ramsden[1], for instance, has shown that simple shaking suffices to precipitate egg-albumin and many other proteids, including the muscle proteids. The latter appear to be particularly ready to fall into insoluble forms, for the change may take place spontaneously, that is without known external cause. The occurrence of such spontaneous precipitation in muscle extracts has long been recognised. Danilewski[2] frequently found his ammonium chloride extracts turbid, or sometimes even containing separated flakes. v. Fürth has called special attention to the phenomenon, which he has used largely in building up his theory of "muscle-clotting." The following quantitative experiments of our own show that even after standing for two days in a cold room a third of the paramyosinogen may have been precipitated from a muscle extract (Exp. 15). In Exp. 16, after standing a week at a low temperature a 5 p.c. magnesium sulphate extract contained neither paramyosinogen nor myosinogen. But apparently the myosinogen had undergone spontaneous clotting in the muscle even before the extract was made. In Exp. 17, after 17 days at a low temperature an extract made with saturated magnesium sulphate, in which paramyosinogen is insoluble, had more than half of the myosinogen precipitated. The formation of this spontaneous precipitate was always accompanied by an increase in acidity. Precipitation occurred, in general, more rapidly in concentrated salt solutions than in weak salt solutions.

---

[1] *Archiv f.* (*Anat. u.*) *Physiol.* S. 517. 1894.

[2] *Zeitsch. f. phys. Chem.* I. S. 158.

	Exp. **15.**		Exp. **16.**		Exp. **17.**			
Temp.	Original extract	After standing 2 days	Original extract	After standing 7 days	Original extract		After standing 17 days	
Air				·0996			·0407	
to 49°	·0641	·0446	·0755	0	0		0	
to 53					0		0	
50°—59°	·0091	·0119	0	0	·0230	} ·0708	·0228	} ·0356
60 —65	·0041	·0148	0	0	·0478		·0128	
66 —100					·0632		·0727	
Total					·1340		·1490	

In Exp. 15, a dog's heart was extracted for 24 hours with 5 volumes of 5 p.c. $MgSO_4$. At the end of that time determinations were made on portions of 50 c.c. of the extract. The remainder was set aside in a cold room (8°—15°) for two days. A large powdery precipitate had now separated; this was insoluble in water and in 5 p.c. $MgSO_4$. The solution had become strongly acid, and had lost much of its hæmoglobin. The precipitate was filtered off and determinations made on the filtrate.

In connection with spontaneous clotting, we may further mention some curious facts, of which we are unable to suggest any explanation.

In Exp. 15, as we have seen, the extract of the heart after standing for two days deposited a precipitate; an extract of the thigh muscles of the same animal, made with the same menstruum, at the same time, and kept in exactly the same way gave no precipitate.

In Exp. 18 the thigh muscles of a dog were extracted with different menstrua under exactly the same conditions, with the following results:

	5 % $MgSO_4$		10 % NaCl		10 % NH_4Cl	
	Paramy.	Myosin.	Paramy.	Myosin.	Paramy.	Myosin.
Thigh	·0725	0	·2203	·1706	·7285	·3652
			$\frac{P}{M}=1·3$		$\frac{P}{M}=1·9$	
Heart	·1325	·1046				
	$\frac{P}{M}=1·27$					

Here the myosinogen had evidently become insoluble in the muscle for the 5 p.c. $MgSO_4$ but not for the other menstrua.

In another case the paramyosinogen had similarly become insoluble in the thigh muscles of the dog for 5 p.c. $MgSO_4$, the analysis giving ·0059 grm. of paramyosinogen to ·1158 grm. of myosinogen.

These examples illustrate sufficiently the possibility of one or both of the characteristic muscle proteids becoming insoluble in the muscle itself, a fact which, of course, emphasises the necessity for caution in judging of the quantitative relations of the proteids in the muscle from their relations in extracts.

2. **The effect of long-continued heating.**

The following quantitative experiments show that by long-continued heating, the whole or a very large proportion both of the paramyosinogen and of the myosinogen may be precipitated in an insoluble form at or below 40°, both in extracts and *in situ* in the muscle.

For shorter periods of heating, such as Halliburton used in certain of the experiments by which he attempted to prove that the myosinogen in extracts of muscle can be caused to clot repeatedly in a still soluble form, true heat precipitation of the proteids also occurs·

No. of Exp.	Temp.	Fresh extract	Heated extract
19	37° for 9 days		·0425
	to 49·5°	·0030	0
	50°—59°	·0573	0
	60 —65	·0139	0
20	37° for 9 days		·0363
	to 49·5°	·0044	0
	50°—59°	·0610	0
	60 —65	·0057	0
21	40° for 5 days		·0440
	to 49·5°	·0755	·0046
	50°—59°	0	0
	60 —65	·0040	0
	65 —100	·0053	·0078
22	45°—49° for 24 hrs		·2230
	to 49° for usual time	·1528	
	50°—59°	·0430	·0087
	60 —65	0	·0126

No. of Exp.	Temp.	Fresh extract		Heated extract	
23	37° for 16 hrs.			·1949*	
	to 48°	·2086		0	
	48°—58°	·0959		·0926	
	60 —80	·0460		·0576	
24	40° for 8 days			·1313	
	to 49·5°	0		0	
	50°—59°	·0230	·0708	·0228	·0349
	60 —65	·0478		·0121	
	65 —100	·0632		·0554	
	non-coag.			·0124	

* by difference.

In Exps. 19, 21, and 22, dog's skeletal muscle was extracted with 5 p.c. $MgSO_4$; in Exp. 23, rabbit's skeletal muscle. In Exp. 20, dog's heart muscle was extracted with 5 p.c. $MgSO_4$. In Exp. 24, dog's skeletal muscle was extracted with a saturated solution of $MgSO_4$, in which paramyosinogen is insoluble. In all the experiments a considerable amount of coagulable proteid was rendered non-coagulable during the prolonged heating, doubtless owing in part to bacterial action, as no precautions were taken to keep the extracts sterile.

Similar results were obtained when the muscle was heated before extraction, as Exp. 25 shows.

Exp. **25.** An extract of the muscles of one thigh of a cat killed a short time before was made by adding 100 parts by volume of 10 p.c. NH_4Cl to 26 parts by weight of muscle. Left in cold for 48 hours, and proteids then determined. The other leg was put into a bath at 42°, and kept there for 48 hours. An extract was then made of the thigh muscles with the same amount of NH_4Cl as was used before. Left in cold for 48 hours, and proteids then determined.

Temp.	Extract of fresh muscle	Extract of heated muscle
45°—48°	·3145	only slight turbidity
55 —58	·015	·039
60 —67	0	·015
67 —100	·004	·019
Total proteid	·3335	·073

v. Fürth obtained results similar to ours, but he does not appear to have succeeded in precipitating the whole of the paramyosinogen or myosinogen, as we have repeatedly done.

3. **Solubility of the muscle proteids in salt solutions and water.**

(1) *The effect on the muscle proteids of saturation with magnesium sulphate.*

Exp. **26** shows the proportion of the total proteid in a 5 p.c. $MgSO_4$ extract of dog's skeletal muscle which is precipitated by the addition of various amounts of $MgSO_4$ up to saturation. Saturation removes the paramyosinogen completely. In column *A* are given the determinations of the heat precipitates in the original extract, in *B* the precipitate caused by the addition of 21 grm. $MgSO_4$ to 80 c.c. of the extract (= 20 p.c. of $MgSO_4$), in *C* the precipitate caused by the addition of 40 grm. $MgSO_4$ to 80 c.c. of the extract (= 33 p.c. of $MgSO_4$), and the heat precipitates in the filtrate, in *D* the precipitate caused by the addition of 80 grm. $MgSO_4$ to 80 c.c. of the extract (= 50 p.c. of $MgSO_4$, making a saturated solution for the temperature worked at in winter), and the heat precipitates in the filtrate.

	A	*B*	*C*	*D*
$MgSO_4$ ↓		·0973	·1525	·3527
Heated to 49·5°	·1999	—	·1219	0
49·5°—59°	·1348	—	·0893	·0505
60 —65	·0684	—	·0521	·0514
65 —100	·0581	—	·0468	·0478
Total	·4612	—	·4626	·5024

Here there was no precipitate up to 49·5° in the filtrate after saturation. Extracts made with saturated solution of magnesium sulphate also show no such precipitate. This is illustrated by Exp. 27, in which dog's skeletal muscle was extracted for the same time with 5 p.c. $MgSO_4$ (*A*), and saturated solution (*B*), in each case 5 parts by volume of the solution to 1 part by weight of the muscle. Although the total amount of proteid extracted by the saturated solution is nearly twice as great as that extracted by the 5 p.c. solution, there is no precipitate in the former up to 49·5°.

Exp.	Temp.	*A*	*B*
27	to 49·5°	·0700	0
	50°—59°	0	·0230
	59 —65	·0015	·0478
	65 —100	·0040	·0632
	Total	·0755	·1340

The effect of saturation of watery extracts of dog's skeletal muscle with magnesium sulphate is shown in Exps. 28 and 29.

In column *A* the heat precipitates yielded by the original watery extract are given, in column *B* the precipitate on saturation and the heat precipitates in the filtrate.

Exp.		*A*	*B*
28	Saturation		·195
	Heat to 48°	·052	·103
	50°—58°	·162	·096
	58 —100	0	0
	Total	·214	·394

Exp.		*A*	*B*
29			·277
		·097	·158
		·232	·142
	60°—62°	·080	·026
	62 —100	·180	·008
	Total	·589	·611

In Exp. 30 the effects of saturating with $MgSO_4$ a 5 p.c. $MgSO_4$ extract and a watery extract of rabbit's skeletal muscle are compared. *A*, heat precipitates of the original 5 p.c. $MgSO_4$ and watery extracts, *B* precipitates after saturation.

Exp.		5 % $MgSO_4$ extract		Watery extract	
		A	*B*	*A*	*B*
30	Saturation		·473		·250
	Heat to 48°	·563	·066	·025	·129
	48°—58°	·441	·289	·293	·073
	58 —62	·032	0	·126	·058
	62 —100	·066	·314	·107	·044
	Total	1·102	1·142	·551	·554
	Difference between totals in *A* and *B*	3·5 %		0·5 %	

(2) *Effect on the muscle proteids of the addition of smaller quantities of magnesium sulphate than is necessary to saturate the extracts.*

Exp. **31** shows the effect of the addition of 50 grm. $MgSO_4$ to 100 c.c. of a 5 p.c. $MgSO_4$ extract of dog's skeletal muscle, a quantity which, according to Halliburton, completely precipitates paramyosinogen. Obviously this is not the case.

	Proteids in 100 c.c. of extract		Proteids in 100 c.c. of ext. precip. by $MgSO_4$		
$MgSO_4$ ↓			·178		
Heat to 48°	·331		·190		
48°—58°	·203	·360	·086	·161	
58 —62	·057		·075		
62 —100	·046		·140		
Total	·637		·669		Difference 4·7 %

Extracts of dog's skeletal muscle made with solutions of magnesium sulphate even stronger than half-saturated contain some paramyosinogen. Thus in Exp. 32, a muscle was extracted with a 42 p.c. $MgSO_4$ solution (50 grm. $MgSO_4$ made up with water to 100 c.c.). The average of the duplicate observations on the amount of the proteids in 100 c.c. of the extract gave for the proteid coagulating at or below 49·5°, ·0651 grm. (maximum difference of duplicates from the mean, ·0028), and for the proteid coagulating between 50° and 61°, ·1163 (maximum difference of duplicates from the mean, ·0068).

(3) *Comparison of the solubility of the muscle proteids in water and in dilute salt solutions.* We attempted to make this comparison, (*a*) by extracting muscle for the same length of time with the same quantity of water and a salt solution, and then determining the proteids in equal volumes of the extracts; (*b*) by dialysing saline extracts of muscle and estimating the proteids as dialysis proceeded. Exps. 33 and 34 are examples of (*a*). In the former dog's, and in the latter rabbit's skeletal muscle was used; in both a 5 p.c. magnesium sulphate extract (*A*)

was compared with an aqueous extract (*B*). It is seen that both paramyosinogen and myosinogen are less easily dissolved out of the muscle by water than by the 5 p.c. magnesium sulphate, but myosinogen is more easily extracted by water than paramyosinogen.

Exp.	Temp.	$MgSO_4$ extract		Water extract	Exp.	$MgSO_4$ extract		Water extract	
33	to 48°	·331		·052	**34**	·563		·025	
	48°—58°	·203	·260	·162		·441	·473	·293	·419
	58 —62	·057		0		·032		·126	
	62 —100	·046		0		·066		·107	
	Total	·637		·214		1·102		·551	
	$\frac{\downarrow \text{ at } 45°—48°}{\downarrow \text{ at } 45°—62°}$	·56		·24		·54		·05	
	$\frac{P}{M}$	1·27		·32		1·19		·05	

As an example of the dialysis experiments we may quote

Exp. **35**, in which a 5 p.c. $MgSO_4$ extract of the thigh muscles of a dog was dialysed for five days (first against running tap-water for two days, and then for three days against distilled water). A heavy precipitate was still given with barium chloride, and the contents of the dialyser still yielded ·138 p.c. of ash. The dialysed extract was filtered and the proteids in it and the original extract estimated.

Exp.	Temp.	Original extract		Extract after dialysis	
35	to 49·5°	·2627		·0175	
	50°—59°	·1079	·2158	·0313	·0501
	59 —65	·1079		·0188	
	65 —100	·0954		·0222	
	Total	·5739		·0898	
	$\frac{\downarrow \text{ at } 40°—49·5°}{\downarrow \text{ at } 40°—65°}$	·55		·27	
	$\frac{P}{M}$	1·21		·34	

We further tested the matter by leaving some chopped muscle for 24 hours in running water, kneading it frequently. It was then covered with water in a beaker and allowed to stand for 24 hours. The water was then filtered off. The filtrate showed only the faintest opalescence on boiling. The residue of the muscle was now covered with 10 p.c. sodium chloride solution and left 24 hours. The filtrate from this extract showed abundance of both paramyosinogen and myosinogen. Both are, therefore, much less soluble in water than in the salt solution.

We may here sum up the conclusions which appear to follow from these quantitative experiments on the solubility of the muscle proteids:

Paramyosinogen is a typical globulin, being completely precipitated by saturation with magnesium sulphate or by dialysis. Where in watery extracts of muscle a precipitate is still caused by heating to 49° after saturation with magnesium sulphate, we are inclined to believe that this precipitate consists not of paramyosinogen but of myosinogen whose coagulation temperature has been depressed by the saturation. For not only is the precipitate at 50° to 59° diminished but also those at 60° to 65° and at 65° to 100°, and the diminution of the precipitate of serum-albumin can only, so far as we see, be explained by a depressing action of saturation on the coagulation temperature. Further, the precipitate obtained by heating to 48° or 49°, may in watery extracts be actually greater in amount after saturation with $MgSO_4$ than before. The addition of magnesium sulphate to 5 p.c. $MgSO_4$ extracts to the amount of 33 p.c. causes the precipitation not of all the paramyosinogen but only of about 40 p.c. of it. Paramyosinogen is only slightly soluble in very dilute saline solutions, such as are formed when muscle is extracted with a large quantity of distilled water. It is probably insoluble in distilled water.

Myosinogen shows the same general properties as regards precipitation, but to a less degree. Saturation with magnesium sulphate precipitates 39 to 69 p.c. of the amount present in a solution, the mean of our experiments being 47 p.c. The addition of an amount of magnesium sulphate equivalent to 33 p.c. precipitates from 30 to 55 p.c. of the myosinogen, and almost as much of paramyosinogen. Myosinogen is only slightly soluble in very dilute solutions of salts, though more soluble than paramyosinogen, since the ratio $\frac{P}{M}$ is conspicuously less in aqueous than in saline extracts of muscle. Probably it is insoluble in distilled water. It is not so readily or so completely precipitated by dialysis as paramyosinogen. This is strikingly shown by the diminution in the ratio $\frac{P}{M}$ as dialysis goes on.

Myosinogen appears to be an atypical globulin occupying an intermediate position between the typical globulins and the albumins. Both proteids are apt to pass "spontaneously" into an insoluble form. The conditions influencing this conversion are imperfectly known. It is, however, favoured by moderate heat, by an acid reaction, and by the presence of proteids already precipitated. It takes place in the muscle itself as well as in extracts.

As regards the solubility of the muscle proteids in solutions of other salts than magnesium sulphate we made only a few quantitative experiments, of which we may cite as an example

Exp. **36.** Dog's thigh muscles were extracted for the same length of time with the same quantity of 5 p.c. $MgSO_4$, 10 p.c. NaCl, and 10 p.c. NH_4Cl (140 grms. of chopped muscle to 700 c.c. of extrahent). Four parallel determinations were made for the proteids in the $MgSO_4$ extract, two for the NaCl extract, and three for the NH_4Cl extract. The averages are given in the table.

Temp.	$MgSO_4$	NaCl		NH_4Cl	
to 49·5°	·0725	·2203		·7285	
50°—59°	0	·1123	·1706	·3440	·3652
59 —65	0	·0583		·0212	
65 —100	0	·0724		·0245	
Total	·0725	·4633		1·1182	
$\frac{\downarrow \text{ at } 40°\text{—}49{\cdot}5°}{\downarrow \text{ at } 40°\text{—}65°}$		·56		·66	
$\frac{P}{M}$		1·29		1·99	

By one of those caprices to which the muscle proteids seem to be liable, spontaneous clotting appears to have taken place in the portion of muscle covered with the magnesium sulphate solutions and not in the others. The residue had a curious "cooked" appearance. The determinations for the magnesium sulphate extract cannot, therefore, be compared with the others. But both paramyosinogen and myosinogen seem to be more soluble in (or at least to be more readily extracted by) 10 p.c. ammonium chloride solution, than in 10 p.c. sodium chloride solution, and paramyosinogen relatively more soluble than myosinogen since the ratio $\frac{P}{M}$ is higher for the ammonium chloride extract than for the sodium chloride extract.

Comparison of our results with the conclusions of previous observers. Halliburton's views on the clotting of the muscle proteids are so well known that we shall not quote them here at length, but shall content ourselves with pointing out in what respects our results differ from his. v. Fürth's conclusions[1] differ radically from Halliburton's in certain important points. His chief results are the following:

1. Paramyosinogen and myosinogen (we translate v. Fürth's terminology

---

[1] *Loc. cit.*

into Halliburton's for the sake of uniformity) are precipitated, very quickly at 40°, more slowly at lower temperatures. The precipitate he terms myosin-fibrin when derived from paramyosinogen, myogen-fibrin when derived from myosinogen. The formation of the precipitate is preceded in the case of myosinogen by the production of a substance with the general properties of paramyosinogen but coagulating by heat at 40°.

2. The precipitate thus formed is insoluble in water and salt solutions.

3. The formation of the precipitate is favoured by many organic substances, and Halliburton's so-called "myosin-ferment" probably belongs to this class and need not be looked on as a specific ferment. No ferment is necessary.

4. Clotting is not accompanied by increase of acidity (in opposition to Halliburton).

5. Paramyosinogen is precipitated from its solutions by dilution. The precipitate, like similar globulin precipitates, is at first soluble in dilute salt solutions, but soon becomes insoluble by being converted into the above-mentioned myosin-fibrin.

6. Both paramyosinogen and myosinogen are precipitated by acids.

7. Kühne and Halliburton's "myosin-clot," whether obtained by allowing cooled plasma to stand or by diluting salted plasma and heating it to 35° to 40°, is insoluble in salt solutions. This is opposed to Halliburton's statement.

8. The serum from this clot contains a considerable amount of paramyosinogen and myosinogen.

9. Halliburton's "myosinogen," prepared by dilution without heating, consists of paramyosinogen precipitated by water. It is soluble in salt solutions but contains no myosinogen.

How do these diverging views stand in the light of our experiments?

1. We can from results already discussed at sufficient length endorse v. Fürth's conclusions 1, 2, 6 and 7 concerning the precipitation of the muscle proteids.

2. Cooled muscle plasma prepared from the dog's thigh muscles by Halliburton's method coagulates in the manner described by that observer. One of us, as already stated, succeeded in obtaining a similar plasma from the heart of the dog and rabbit, in the form of a yellowish, alkaline liquid coagulating on heating at the same temperatures as plasma prepared from skeletal muscle. It did not always clot at the ordinary temperature, but did so, without dilution, when kept at about 38° for 15 to 20 hours.

3. The clot in either case is never entirely soluble in 5 p.c.

magnesium sulphate. Whether any, or how much, goes into solution we have not determined with sufficient accuracy.

4. Salted muscle plasma, prepared by extraction either with 5 p.c. magnesium sulphate or 10 p.c. sodium chloride, clots when kept at about 35°. Clotting is more prompt if the extract is first diluted with 3 to 5 volumes of water, but it is also obtained without dilution. It may very often, but not always, occur at room temperature. The clot is insoluble in 5 p.c. magnesium sulphate.

5. The composition of the serum, as regards proteids, depends on the time of heating and other circumstances. Under some conditions it may not give any precipitate below 73°. When obtained in the manner described by Halliburton, as nearly as we could follow his directions, all the muscle proteids were represented in it.

6. During the clotting there is an increase of acidity.

As much emphasis has been laid on this by Halliburton, and as it has been not less emphatically denied, we subjoin the protocol of a typical experi-

	Quantity of NaOH $\frac{N}{10}$ needed for neutralisation in c.c.
5 c.c. 1 hour after death	·1
,, left at air temperature for 1 day, no ↓	·1
,, ,, ,, ,, 2 days, slight ↓	·15
,, left at 35° for 16 hours, flaky ↓ in less than 16 hours	·25
,, ,, ,, 41 ,, more ↓	·6
,, +phenol phthalein + 20 c.c. H_2O, left at 35° for 16 hours, flaky ↓ in less than 16 hours	·45
,, ,, ,, ,, ,, left at 35° for 41 hours	·4
,, ,, ,, ,, ,, + ·1 c.c. NaHO $\frac{N}{10}$ at 35° for 4½ hours, colour discharged in 2 hours, flaky ↓ in 4½ hours	·35*
,, ,, ,, ,, ,, + ·2 c.c. NaHO $\frac{N}{10}$ at 35° for 16 hours, colour discharged, flaky ↓	·4*
,, ,, ,, ,, ,, + ·5 c.c. NaHO $\frac{N}{10}$ left at 35° for 41 hours, colour discharged in 19 hours, flaked in 21 hours	·6*
,, ,, ,, ,, ,, + 2 c.c. NaHO $\frac{N}{10}$ at 35° for 48 hours, colour not discharged, flaked in less than 16 hours†	
,, ,, ,, ,, ,, + 5 c.c. NaHO $\frac{N}{10}$ at 35° for 48 hours, colour not discharged, flaked in 20 hours†	

* Including what was added before heating.

† Precipitate insoluble in dilute acids, and gives faint xanthoproteic reaction.

ment in which the acidity was determined under various conditions in a muscle extract by titration with decinormal sodium hydrate solution, phenol phthalein being used as indicator. The extract was made from the skeletal muscles of a pup eight weeks old, killed by bleeding from the carotid. Half-an-hour after death they were hacked, then rubbed up with sand and 10 p.c. NaCl solution, strained and filtered. The filtrate was perfectly colourless and slightly turbid. Separate portions of 5 c.c. of the filtrate were taken for each titration.

7. The clot consists often of bulky flakes and not of a continuous coagulum. On the other hand undoubted heat precipitates may take the form of a continuous oscillating jelly. So that no fundamental distinction between "clotting" and other forms of precipitation can be based on the appearance of the clot.

8. The clot or precipitate is not readily redissolved by putrefaction. We have seen it remain intact for four months.

9. The precipitation produced by Halliburton by dilution and heating to 35° is, as v. Fürth has asserted, identical with "spontaneous clotting," *i.e.* the formation of v. Fürth's myosin-fibrin. The "clot" formed on the addition of water in the cold is probably a dilution precipitate of paramyosinogen and myosinogen.

10. Extracts freed from paramyosinogen clot, but more slowly than when the paramyosinogen is present. (In certain experiments clotting took place before removal of the paramyosinogen in less than 20 minutes; after removal, in 80 to 200 minutes.)

11. As already stated we did not succeed in preparing pure paramyosinogen artificially. But we encountered in the course of our work extracts (especially from the heart) which contained paramyosinogen but no myosinogen. Abundant clots formed in these.

12. The clotting of muscle-plasma or muscle-extracts, or solutions containing either paramyosinogen or myosinogen alone, consists in the partial or complete conversion of these proteids into an insoluble modification having the general properties of heat-coagulated proteid.

As regards the terminology of the muscle proteids, v. Fürth has introduced a new nomenclature in which he employs certain of the current names for entirely different substances. This cannot fail to breed confusion, and we would suggest that Halliburton's terms should be retained with the alteration which appears necessary in view of the fact that myoglobulin has hardly made good its claim to an independent existence. Since the only well-established difference between v. Fürth's soluble myo-fibrin and his myosin seems, so far, to be a difference of a few degrees in the temperature of heat coagulation, we suggest that provisionally they be grouped as one. Thus

Coagulation temp.	Halliburton	Fürth	Authors
40°	—	Soluble myo-fibrin	Paramyosinogen
about 46°	Paramyosingen	Myosin	
,, 56	Myosinogen	Myogen	Myosinogen
,, 63	Myoglobulin		
Spontaneous coagulum	Myosin	Myosin-fibrin or Myo-fibrin	Myosin

Quantity of various proteids in muscle.

As to the quantity of paramyosinogen and myosinogen in muscle and its variations under different conditions, observations have been previously made mainly by Demant and Danilewski. Demant[1] simply extracted muscle with distilled water until the extracts ceased to give a precipitate at 48°, and then determined the amount of the substance coagulating at that temperature in his extracts. We have shown that muscle completely exhausted with water still gives up to 10 p.c. ammonium chloride a large quantity of proteid coagulating at or below 49·5°, so that Demant's assumption that he had extracted the whole of this substance is incorrect, and his quantitative determinations and the rather broad generalizations[2] which he bases on them lose a great part of their value.

Danilewski[3] endeavoured to estimate the quantity of myosinogen by extracting muscle with 10 p.c. ammonium chloride after previous exhaustion with water. Here the result evidently depends on the amount of proteid removed by the water, and, as we have seen, a certain quantity both of paramyosingen and of myosinogen is dissolved out of muscle by water by the aid of the salts present in the muscle itself. Further, the tendency of the muscle proteids to become insoluble in the muscle renders the proof that *all* of a given proteid has been extracted exceedingly difficult, and the longer the extraction has been continued the more likely is it that spontaneous clotting will have taken place.

---

[1] *Zeitsch. f. physiol. Chem.* III. S. 241.

[2] For instance, that the muscles which work hardest contain the greatest amount, that there is more in adult than in young animals, more in certain kinds of animals than in others, and none at all in the heart. We found both in the skeletal muscles of the embryo and the adult heart in general more paramyosinogen than myosinogen, always abundance.

[3] *Ibid.* v. S. 158. 1881.

For these reasons we did not attempt to completely exhaust the muscle, but confined ourselves to determining the proportional amounts of the various proteids in extracts made under given conditions. This enabled us for one thing to shorten the time of extraction (in some experiments even to half-an-hour or an hour), and therefore to lessen the risk of spontaneous clotting. But we do not pretend to be able to say with absolute certainty that in a given extract the relative amount of paramyosinogen and myosinogen must be the same as in the muscle. It is certainly not indifferent what liquid is used for the extraction. Water, for instance (Exp. 30), is a worse solvent for paramyosinogen than 5 p.c. $MgSO_4$ solution, and this is probably the case also with normal saline solution, which v. Fürth used in his experiments. This seems to be one reason at any rate why the ratio P : M, as deduced from his results, for instance ·3, ·23, ·2, respectively in three experiments is so much lower than we obtained. Since of the two chief proteids paramyosinogen is, in general, the more insoluble we assumed that the liquid in which its solubility as compared with that of myosinogen is greatest, viz. 5 p.c. magnesium sulphate, would give more correct results than any other. To test the matter we made repeated extractions, with the expectation that if we were really dissolving out the proteids in the relative amount in which they exist in muscle, the ratio P : M should be approximately the same in the last extract as in the first, if spontaneous clotting in the muscle did not take place. We were prepared, however, to find instances in which the paramyosinogen might undergo spontaneous precipitation during the extraction while the myosinogen remained soluble or was precipitated to a smaller extent, in which case P : M must diminish in the successive extracts. It was possible that in other cases the opposite might occur, the myosinogen being spontaneously precipitated to a greater extent than the paramyosinogen; and then the ratio P : M must increase. As a matter of fact these three conditions are all represented in our results, Exp. 37 being an example in which the ratio altered but little in the extracts of skeletal muscle while it increased in the extracts of heart muscle. In Exp. 38 the ratio markedly diminished in the extracts of heart muscle; in the thigh muscles the paramyosinogen seemed to be practically insoluble from the start. The fact that the increase or diminution was not gradual but abrupt seems to us to be strong evidence that it was not due to the complete extraction of one or other of the proteids from the muscle, but to a change which rendered it insoluble.

		Skeletal muscle (thigh)				Heart muscle		
Exp.	Temp.	1st ext.	2nd ext. A	2nd ext. B		1st ext.	2nd ext.	
37	to 49°	·1526	·0803	·1124		·0543	·0825	
	49°—59°	·0430	·0244	·0252	·0377	·0357	·0157	·0208
	59 —65	0	·0008	·0125		0	·0051	
	P : M	3·55	3·18	2·98		1·52	3·96	

All the extracts were made with 5 p.c. $MgSO_4$. The first extract of skeletal muscle was allowed to stand 24 hours, then filtered, and the determination made on the filtrate. The residue was extracted with another portion of $MgSO_4$ for three days, filtered without squeezing, and the proteids in the filtrate determined (second ext. A). The residue was then squeezed out and filtered and the proteids determined in this filtrate (second ext. B). In the second extract of the heart the filtrates before and after squeezing were mixed, and the proteids estimated in the mixture.

		Skeletal muscle					Heart muscle				
Exp.	Temp.	1st ext.	2nd	3rd	4th	5th	1st ext.	2nd	3rd	4th	5th
38	to 49·5°	·0057	0	·0030	trace	·0028	·0649	0	·0044	trace	·0025
	50°—59°	·0557	·0591	·0573	·0355	·0364	·0272	·1330	·0610	·0370	·0656
	59 —65	·0102	·0280	·0139			·0173	·0114	·0057		
	50 —65	·0659	·0871	·0742	·0355	·0364	·0445	·1444	·0667	·0370	·0656

The muscle (dog's thigh muscles and heart) was extracted with 5 p.c. $MgSO_4$, for the 1st extract for 24 hours.

The extract was filtered off, the residue covered with the same amount of 5 p.c. $MgSO_4$ solution as was used before, and allowed to stand 4 days (2nd extract). The residue was again extracted with a fresh portion of $MgSO_4$ solution for 3 days (3rd extract); then for 7 days (4th extract); then for 5 days (5th extract). The temperature of the room in which the extracts stood was during all this time only a degree or two above freezing-point.

These and other experiments already quoted show that the conditions which determine the amount of the muscle proteids which can be extracted by a given solvent are complex, and that the amount does not depend merely on the quantity present in the muscle. A muscle which for the first day of extraction may yield a large amount of paramyosinogen to the extrahent, may on the second day refuse to yield any, not because it is exhausted of paramyosinogen but because this substance has assumed an insoluble form. Since we know but little of the conditions that favour this spontaneous clotting and can therefore only to a limited extent control it, there is great difficulty in drawing correct deductions as to the quantitative relations of the muscle proteïds. The following conclusions, however, appear to follow from our observations.

1. It is practically impossible to remove all the proteids from muscle by repeated extraction with saline solutions, the time required for their complete solution being so great that spontaneous clotting can hardly be excluded.

2. Partial extraction is a better method for determining the relative proportions of muscle proteids, although certain sources of error have also to be taken account of:

(*a*) Paramyosinogen being less soluble than myosinogen, partial extraction will yield solutions in which the ratio P : M is too low.

(*b*) Since paramyosinogen has a greater tendency to undergo spontaneous clotting than myosinogen, and may become partially insoluble from this cause during or even before extraction, the ratio P : M in our extracts is likely to be too low rather than too high.

3. Notwithstanding the influences mentioned in (*a*) and (*b*), which tend to depress the ratio P : M, we find that on the average there is in muscle extracts made with 5 p.c. magnesium sulphate, 10 p.c. sodium chloride and 10 p.c. ammonium chloride, twice as much paramyosinogen as myosinogen. A synopsis is given in the following table of the values of this ratio in 27 experiments on skeletal muscle (thigh muscles) and nine on heart muscle.

	Skeletal muscle					Heart				
Animal	No. of exps.	Average P : M[1]	No. of exps. with P : M<1	No. of exps. with no P	No. of exps. with no M	No. of exps.	Average	P : M<1	No P	No M
Dog	16	1·83	4	2	1	6	2·04	1	0	0
Rabbit	6	3·17	0	0	0	1	—	—	—	1
Cat~~[2]~~	1				1	—	—	—	—	—
Frog[2]	4	2·53	1	1	0	2	3	—	—	1
All	27	2·31	5	3	2	9	2·17	1	0	2

Halliburton states that myosinogen was more abundant than paramyosinogen in his muscle extracts, but this statement was based on qualitative experiments. v. Fürth found far smaller values for the ratio P : M than we have done. We have already explained this as due to his use of normal saline solution for extraction.

4. We found no constant differences as to the quantitative

---

[1] Omitting experiments in which one or other of the two proteids was absent from the extract.

[2] The extracts used for the estimation of the proteids in the frog's heart were made in one case from 50, and in the other from 20 hearts.

relations of the two proteids between the muscles of different animals[1], nor between the heart and skeletal muscles of the same animal.

Bottazzi and Ducceschi[2] state that heart muscle contains less myosinogen and paramyosinogen than skeletal muscle, and a proportionally large amount of nucleo-proteid. We believe, in view of the sources of error discussed above, that a large number of determinations would be needed to render such a conclusion safe.

5. Nor were any constant differences in the ratio P : M made out between muscles in commencing rigor, in maximum rigor, and after rigor had passed off in the absence of recognisable putrefactive changes.

Bearing of these results on theories of rigor mortis.

Conclusion 5 would seem to indicate that the aseptic resolution of rigor is not due to the digestion by a proteolytic ferment of the muscle proteids, as has been suggested. It was noticed, however, that extracts made after the passing off of rigor, although they contained abundance of paramyosinogen and myosinogen, did not show any trace of coagulation when diluted and kept for 20 hours at 32°—34°. This might indicate that a radical change had taken place in the coagulation properties of the muscle proteids. Yet as regards heat coagulation at temperatures above 40° the extracts behaved just like extracts of the fresh muscle.

The fact that the precipitates which come down in muscle extracts either "spontaneously" at ordinary temperatures or during prolonged heating at temperatures below 40°, are by no means easily altered by putrefactive bacteria, suggests either that the clot which forms within the muscle in natural rigor has different properties from the "clot" produced in extracts, or that putrefaction is not essentially concerned in the resolution of rigor. We are not able at present to offer a theory of the resolution of rigor which will take account of all the facts, and we doubt whether recent work has not rendered the process more mysterious than ever.

As to the onset of natural rigor, the theory, associated with the names of Virchow, Vulpian, Schiff, Brown-Séquard, etc., that it is the last vital act of the muscle, and, therefore, analogous to a permanent contraction or contracture, does not appear to be well-grounded, although it is necessary to suppose that under some conditions, as for example when skeletal muscle is rapidly killed by heat or by fixing reagents, or when the muscular walls of the heart or the hollow viscera remain tonically contracted after the circulation has ceased, the process of rigor can surprise the muscle, so to say, in the contracted condition and fix it there.

---

[1] Except that on the average the ratio P : M was higher in the rabbit than in the dog. But this might as easily have been due to a difference in the solubility of the proteids as to a difference in the quantity present in the muscle.

[2] *Centralb. f. Physiol.* XII. S. 9.

The theory of Kühne that the proteids are precipitated in muscle after death, as they are in extracts of the muscle, and that the essential phenomena of rigor are due to this precipitation, has many facts in its favour, although the mechanism by which this precipitation is determined in natural rigor is far from clear. If we suppose that during life the main portion of the proteid constituents of muscle consists of paramyosinogen and myosinogen with the same properties as those bodies exhibit in artificial extracts, it is difficult to understand why they are not precipitated in view of their marked tendency to pass into an insoluble form, nor why, if rigor is dependent upon such precipitation, the precipitate should redissolve *in situ* but not in artificial extracts. It was with the hope of casting light upon this problem that this research was originally undertaken. It had been noticed that a higher temperature was required to cause heat-rigor in certain muscles than in others. For instance, the frog's heart may be heated for a short time to a temperature two or three degrees above that which suffices to kill the gastrocnemius; the muscles of mammals can in general resist a temperature several degrees above the limit which is fatal to the muscles of the frog; a fresh muscle can stand a higher temperature than a fatigued muscle. The question naturally suggested itself: Do those differences in the temperature necessary for heat-rigor depend on differences in the heat-coagulation temperature of the muscle proteids which can be extracted from the different muscles? It was evident that an affirmative answer to this question would afford strong proof of the existence of the proteids of the extracts as such in the living muscle. The answer, however, as the above results show, was in the negative. We are, therefore, inclined to believe that paramyosinogen does not exist as such in the living muscle. The heat-coagulation temperature of myosinogen lies far above the temperature necessary for heat-rigor, and therefore these investigations do not indicate whether it is ready-formed in the muscle or not. However this may be, it must be assumed, we think, that after death paramyosinogen and myosinogen are precipitated in the muscle partly in an insoluble form. In heat-rigor, produced by a temperature below the heat-coagulation temperature of paramyosinogen, this is also the case. For instance, a 10 p.c. sodium chloride extract of the skeletal muscles of a frog which had been heated for 2 hours and 20 minutes at a temperature slowly rising from 20° to 37° before extraction, contained only half as much myosinogen and less than one-third as much paramyosinogen as a similar extract of the unheated muscles. The proteids are not, however, completely precipitated, or at any rate the whole of the paramyosinogen and myosinogen is not rendered insoluble, for saline solutions usually extract considerable quantities of both from dead and rigid muscle. In like manner the whole of these proteids is never precipitated from artificial extracts at the ordinary temperature, nor, except with very prolonged heating, at temperatures below 40°.

SUMMARY OF CHIEF CONCLUSIONS.

1. The method of fractional heat precipitation can be usefully employed in the quantitative investigation of the muscle proteids. It is open however to certain important sources of error which cannot always be completely eliminated or controlled, and the results obtained must, therefore, be interpreted in the light of the characteristic properties of the proteids as determined by other methods.

2. There exist in dead muscle two proteids, a true globulin, paramyosinogen, coagulating at about 45° to 50°, and an atypical globulin, myosinogen, coagulating at about 50° to 65°. The latter readily passes into a modification very similar to, if not identical with, the former.

3. Both paramyosinogen and myosinogen pass readily into an insoluble modification myosin. This transformation may take place in the dead muscle itself as well as in extracts.

4. In general paramyosinogen seems to be more abundant in dead muscle than myosinogen, or at least more of it is extracted by saline solutions like 5 p.c. magnesium sulphate.

5. Paramyosinogen is less soluble than myosinogen in very dilute saline solutions (*e.g.* extracts of muscle made with water contain relatively more myosinogen than paramyosinogen).

6. No marked quantitative differences in the relative amount of the two proteids could be established in the muscles of different animals, in different muscles of the same animal, or in rigor mortis.

But the method of fractional heat-coagulation, even with all the precautions we could think of, is incapable, in its present form, of detecting minute differences with certainty.

7. While the~~s~~re are facts which indicate that rigor mortis is associated with the precipitation of paramyosinogen and myosinogen, the properties of the proteids of muscle extracts do not afford a complete explanation of the production and resolution of rigor. Since paramyosinogen is less soluble than myosinogen and more readily undergoes spontaneous coagulation, it is a possible hypothesis, though nothing more, that at the death of the muscle the onset of rigor is determined by the formation of paramyosinogen from myosinogen or the formation of both from some other body.